Breast Cancer Disparities: Ancestry and Biological Determinants

Melissa B Davis, PhD
Scientific Director of ICSBCS
Assistant Prof. of Cell and Developmental Biology in Surgery
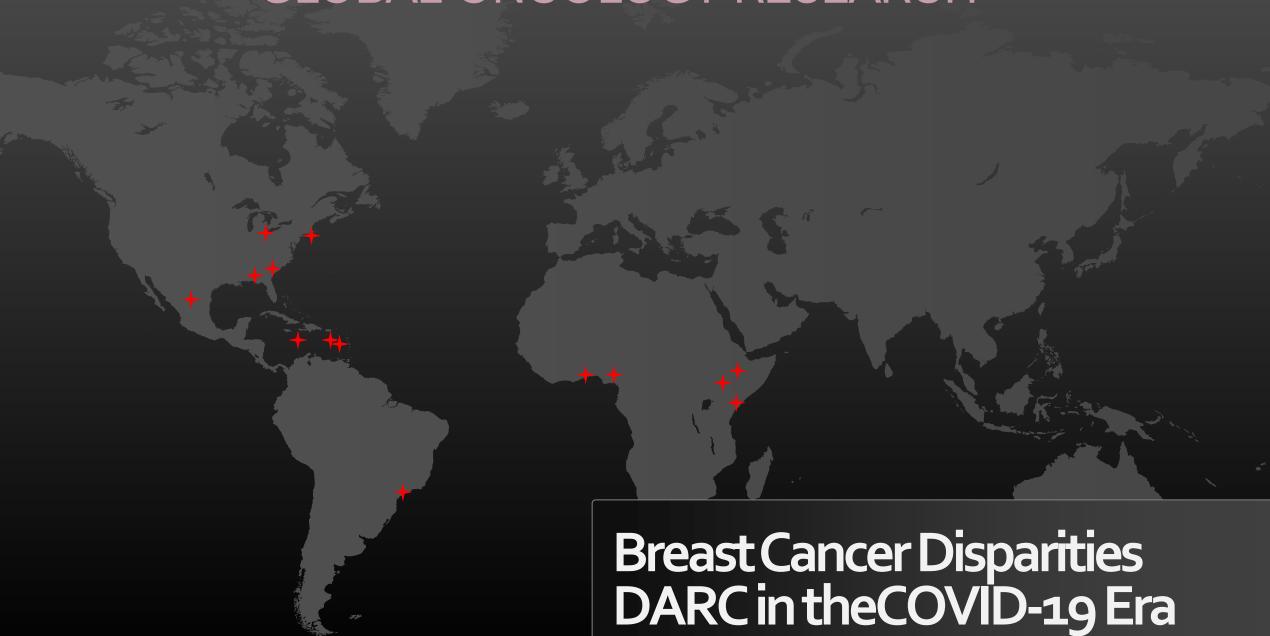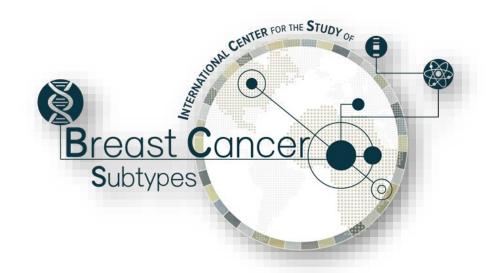Weill Cornell Medicine

Melissa B Davis, PhD

I have no disclosures

Outline of "Stories"

Topics to Discuss

- Emergence of Disparities and Unmasking Tumor Biology

- Population – Private Risk Alleles
  - Previous race-associated risk
  - BRCA1/2 in Africans

- Race vs Ancestry in Gene Network Changes
  - Cancer Pathways (altered)
  - Immune response (altered)

- Tumor Immunology – Evolution at Work
  - DARC phenotype?
  - Novel tumor cell

- COVID-19 Disparities – the DARC connection (proposal)

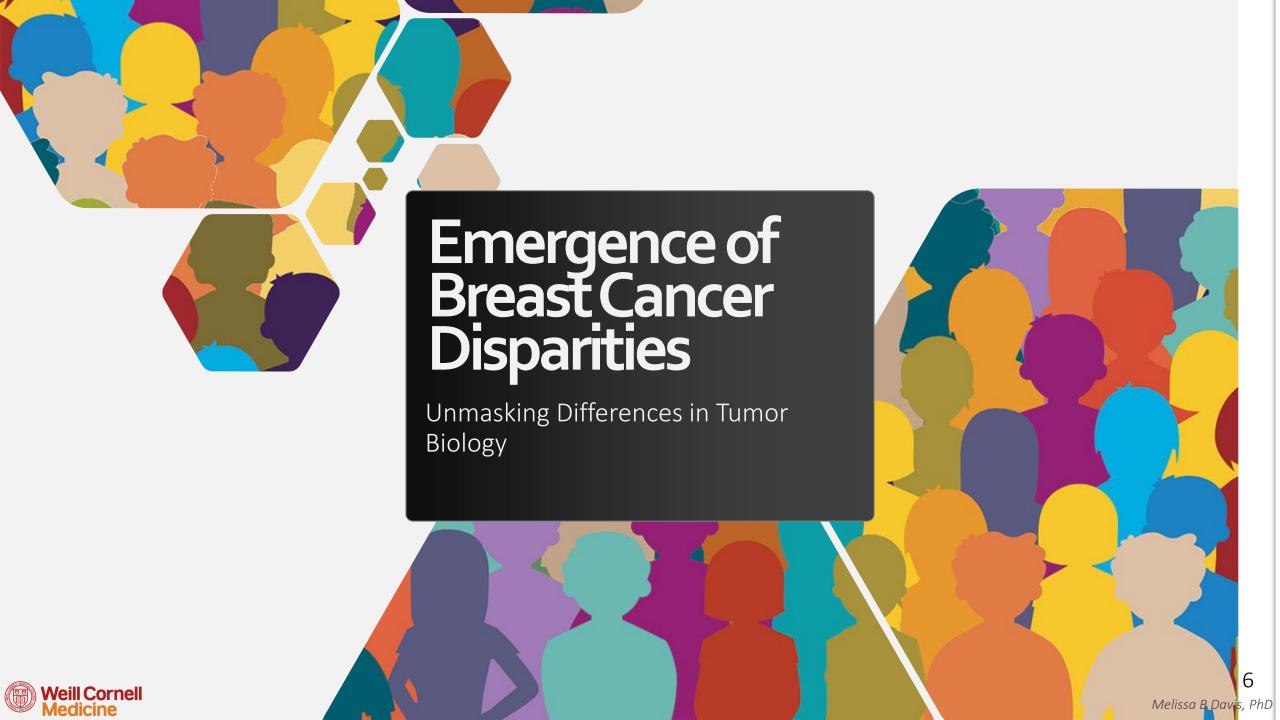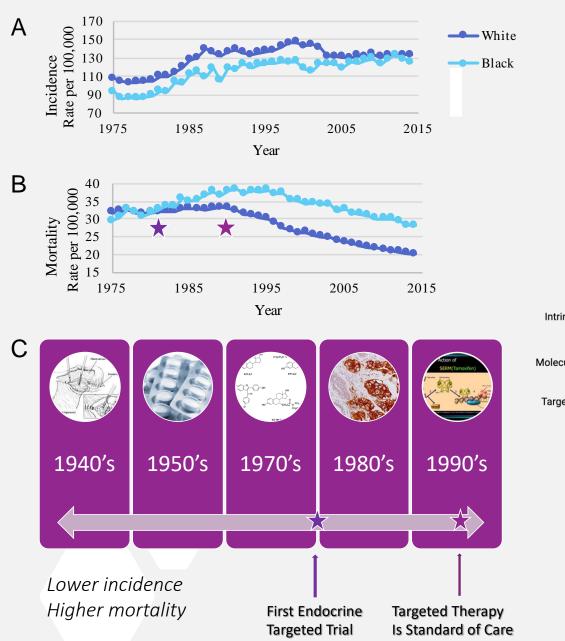- Impact of COVID-19 in cancer patients (emerging... and variable)

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Breast cancer mortality is more than

40%

higher for African American women in the U.S. than Caucasian women.

SUSAN G. KOMEN.
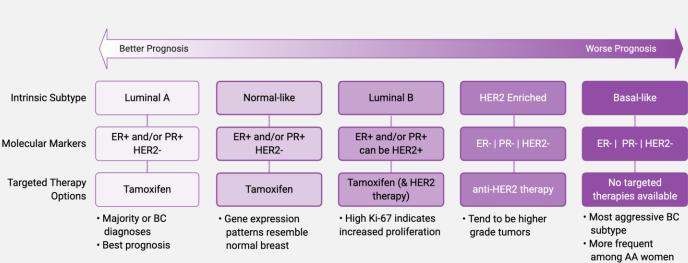
Background of Breast Cancer Disparities

Emergence of biased outcomes

Emergence of Breast Cancer Disparities

Unmasking Differences in Tumor Biology

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Emergence of Survival Disparity

A

Incidence Rate per 100,000 — plotted by Year (1975–2015), legend: White, Black

B

Mortality Rate per 100,000 — plotted by Year (1975–2015)

C

| 1940's | 1950's | 1970's | 1980's | 1990's |

Lower incidence
Higher mortality

First Endocrine Targeted Trial

Targeted Therapy Is Standard of Care

Better Prognosis → Worse Prognosis

Intrinsic Subtype	Luminal A	Normal-like	Luminal B	HER2 Enriched	Basal-like
Molecular Markers	ER+ and/or PR+ HER2-	ER+ and/or PR+ HER2-	ER+ and/or PR+ can be HER2+	ER- | PR- | HER2-	ER- | PR- | HER2-
Targeted Therapy Options	Tamoxifen	Tamoxifen	Tamoxifen (& HER2 therapy)	anti-HER2 therapy	No targeted therapies available

- Majority or BC diagnoses
- Best prognosis

- Gene expression patterns resemble normal breast

- High Ki-67 indicates increased proliferation

- Tend to be higher grade tumors

- Most aggressive BC subtype
- More frequent among AA women
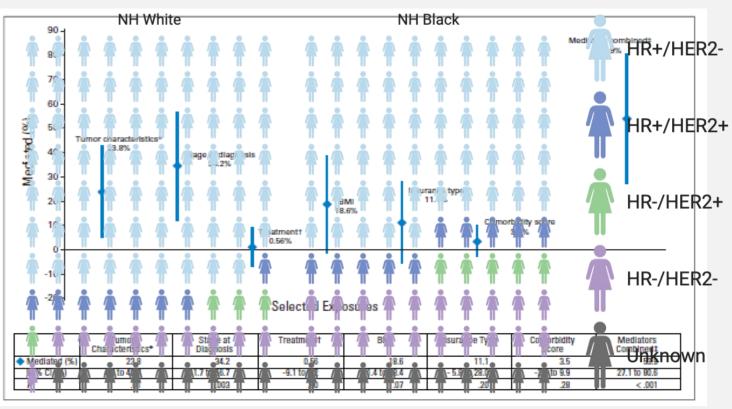
Racial Differences in Survival – Contemporary Mediating Effectors

Warner ET, Tamimi RM, Hughes ME, et al. Racial and Ethnic Differences in Breast Cancer Survival: Mediating Effect of Tumor Characteristics and Sociodemographic and Treatment Factors. J Clin Oncol. 2015;33(20):2254–2261. doi:10.1200/JCO.2014.57.1349

Distribution of BC Subtypes among non-Hispanic White and non-Hispanic Black women in the US, 2012-2016. Data from the SEER program and CDC National Program of Cancer Registries, statistics reported from DeSantis et al 2019. (Breast Cancer Statistics, 2019)

Global Disparities

The Imprint of an African Diaspora

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Global perspectives of Breast Cancer Incidence and Mortality

Number of new breast cancer cases occurring worldwide, per 100,000.

Global TNBC Frequencies

USA TNBC Frequencies by Race/Ethnic groups

Newman et al 2019

BREAST CANCER
Mortality rate per 100,000, female

- 18.1+
- 15.0-18.1
- 13.1-15.0
- 10.1-13.1
- <10.1
- No Data
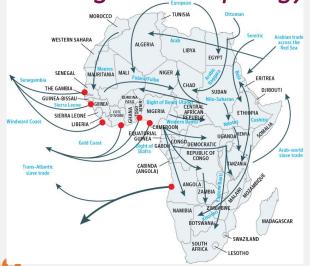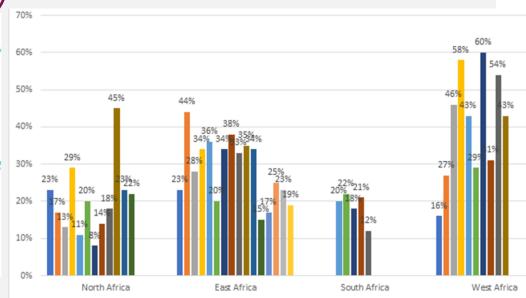
Africa has lowest incidence, but highest mortality burden *Adapted from WHO data*

Oncologic Anthropology

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Could our ancestral African genes play a role in cancer biology?

THE AFRICAN CONNECTION

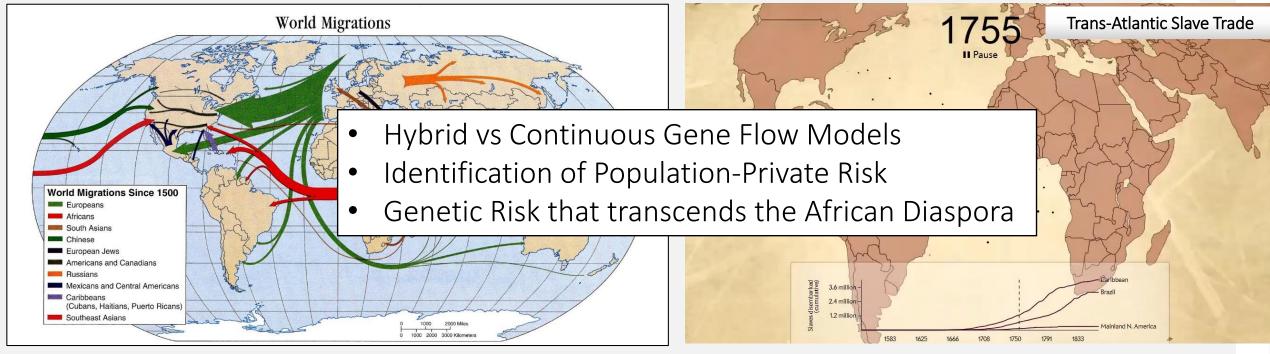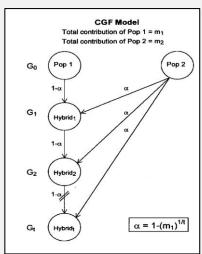# Global Human Populations and African Ancestry

World Migrations

World Migrations Since 1500
- Europeans
- Africans
- South Asians
- Chinese
- European Jews
- Americans and Canadians
- Russians
- Mexicans and Central Americans
- Caribbeans (Cubans, Haitians, Puerto Ricans)
- Southeast Asians

Trans-Atlantic Slave Trade

1755
⏸ Pause

- Hybrid vs Continuous Gene Flow Models
- Identification of Population-Private Risk
- Genetic Risk that transcends the African Diaspora

CGF Model
Total contribution of Pop 1 = m_1
Total contribution of Pop 2 = m_2

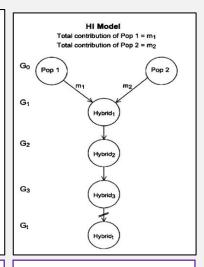$\alpha = 1-(m_1)^{1/t}$

HI Model
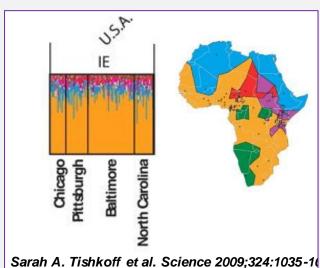Total contribution of Pop 1 = m_1
Total contribution of Pop 2 = m_2

Influx of parental (African) alleles
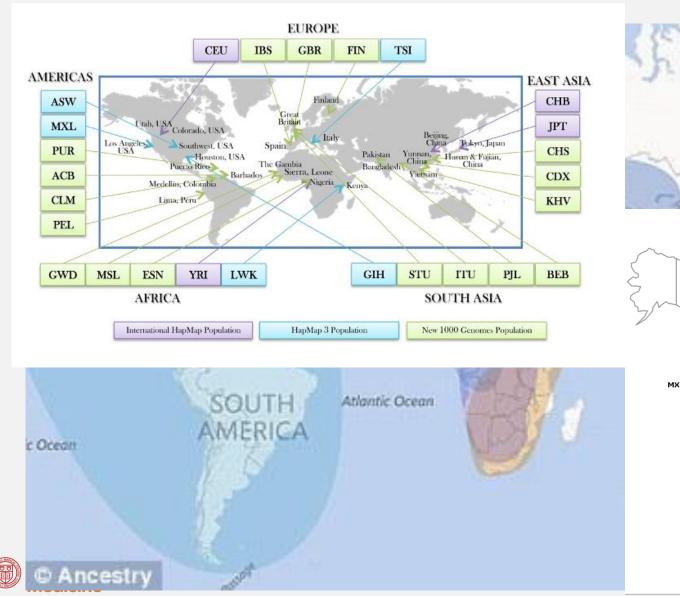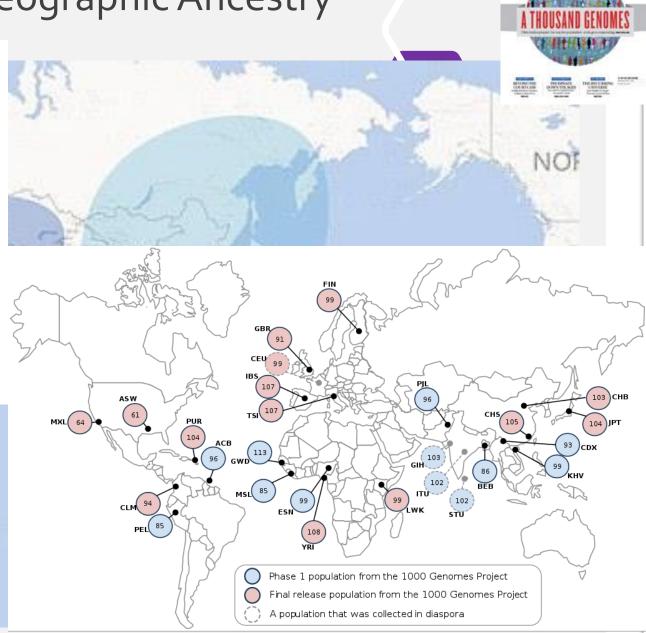
Recombination of the founder alleles + drift

Sarah A. Tishkoff et al. Science 2009;324:1035-1

Overview of the Slave Trade Out of Africa
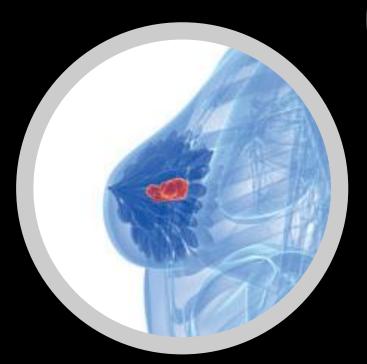Number of slaves

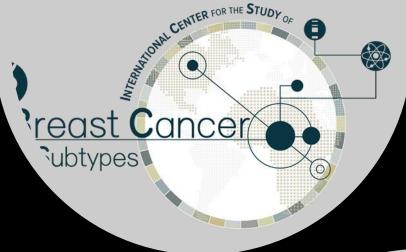# Ancestrally Informative Markers Geographic Ancestry
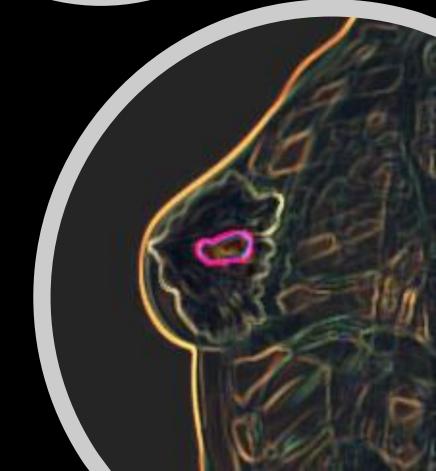
Differences in TNBC Tumor Biology
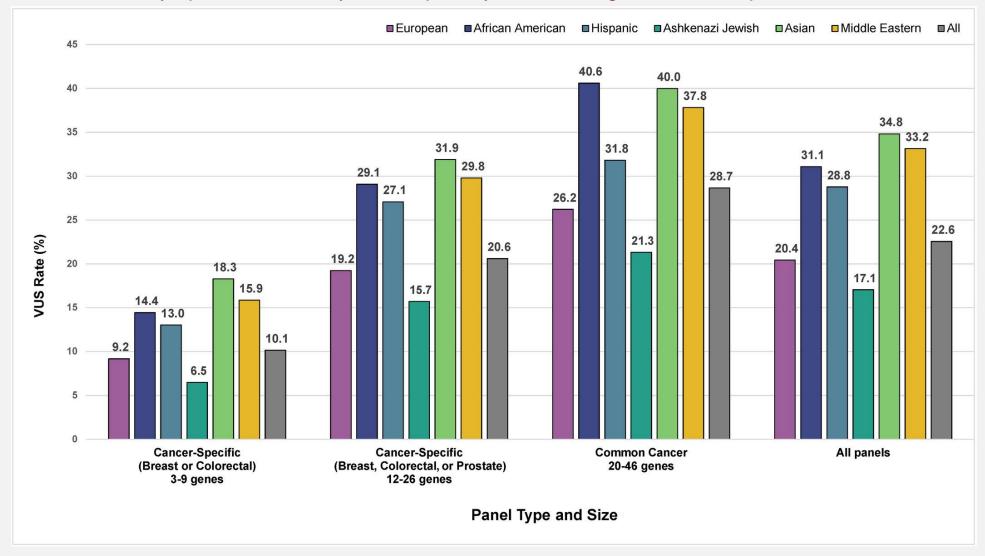
Emerging Evidence

Population-private Risk Alleles

Power of Inclusion

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Ancestry-specific hereditary cancer panel yields: Moving toward more personalized risk

Weill Cornell Medicine

Hereditary Susceptibility for Triple Negative Breast Cancer Associated With Western Sub-Saharan African Ancestry

Results From an International Surgical Breast Cancer Collaborative

Lisa A. Newman, MD, MPH,*✉ Brittany Jenkins, PhDc,* Yalei Chen, PhD,† Joseph K. Oppong, MBChB,‡
Ernest Adjei, MBChB,‡ Aisha S. Jibril, MD,§ Syed Hoda, MD,* Esther Cheng, DO,* Dhananjay Chitale, MD,‡
Jessica M. Bensenhaver, MD,‡ Baffour Awuah, MBChB,‡ Mahteme Bekele, MD,§ Engida Abebe, MD,§
Ishmael Kyei, MBChB,‡ Frances Aitpillah, MBChB,‡ Michael Adinku, MBChB,‡ Saul David Nathanson, MD,†
LaToya Jackson,† Evelyn Jiagge, MD, PhD,¶ Sofia Merajver, MD, PhD,¶ Lindsay F. Petersen, MD,†
Erica Proctor, MD,† Kofi K. Gyan, DVM, MPH,* Rachel Martini, PhDc,*
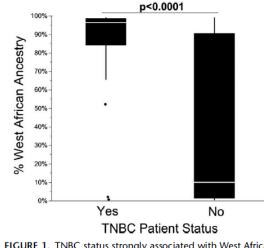Rick Kittles, PhD,‖ and Melissa B. Davis, PhD*

FIGURE 1. TNBC status strongly associated with West African ancestry. TNBC patient status compared to percent West African ancestry (n = 82; TNBC yes = 44, TNBC no = 38; Student *t* test, *P* < 0.0001, CI [−0.6078, −0.3066]).

			Models With Covariates*									
			By SNV Genotypes Weighted (Dominant Model)					By SNV Alleles Weighted (Dosage model)				
SNV ID	**Minor Allele**	**Sample Size**	**OR**	**SE**	**Lower 95% CI**	**Upper 95% CI**	**P Value**	**OR2**	**SE2**	**Lower 95% CI2**	**Upper 95% CI2**	**P Value**
rs2814778	C	297	0.283	0.896	0.049	1.637	0.0473	0.782	0.346	0.397	1.54	0.000345
rs13000023	A	50	0.077	1.187	0.008	0.790	0.0308	0.258	0.691	0.067	0.9998	0.04996
rs2363956	G	43	0.106	1.271	0.009	1.278	0.0773	0.132	0.955	0.020	0.855	0.03369
rs2981578	C	50	0.778	1.215	0.072	8.415	0.8362	0.778	1.215	0.072	8.415	0.8362
rs2981579	G	50	1.121	1.254	0.096	13.090	0.9271	0.882	0.727	0.212	3.669	0.8631
rs3112572	A	44	0.746	0.895	0.129	4.313	0.7438	0.941	0.764	0.210	4.205	0.936
rs3745185	A	45	0.813	1.014	0.111	5.932	0.8378	0.813	1.014	0.111	5.932	0.8378
rs4245739	C	49	0.718	0.869	0.131	3.944	0.7032	0.469	0.632	0.136	1.620	0.2315
rs4849887	T	48	6.725	1.207	0.631	71.680	0.1145	3.057	0.862	0.564	16.560	0.1949
rs609275	C	50	0.562	0.931	0.091	3.483	0.5357	0.562	0.931	0.0907	3.483	0.5357

*rs2814778 was adjusted for race and age while all other SNVs were adjusted for race.
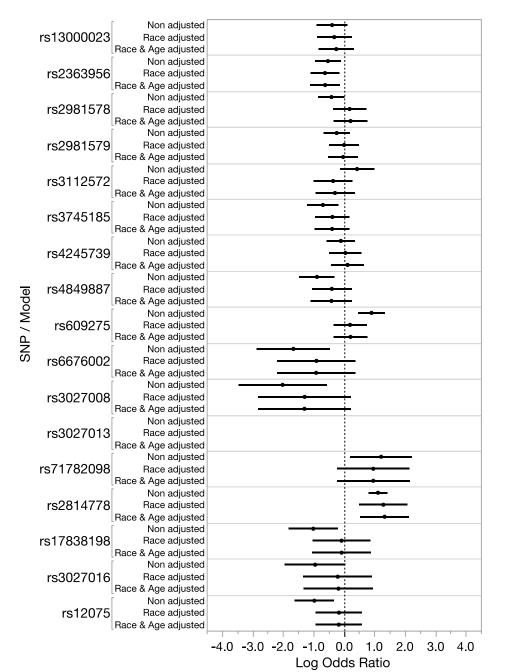CI indicates confidence interval; OR, odds ratio; SE, standard error; SNV, single-nucleotide variant.
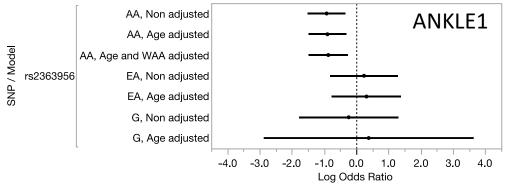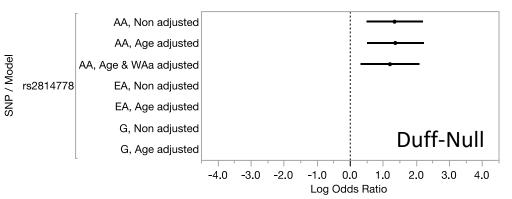Dominant Model: evaluates association between homozygous minor allele status and breast cancer risk.
Dosage Model: evaluates association between presence of the minor allele (whether homozygous or heterozygous) and breast cancer risk.

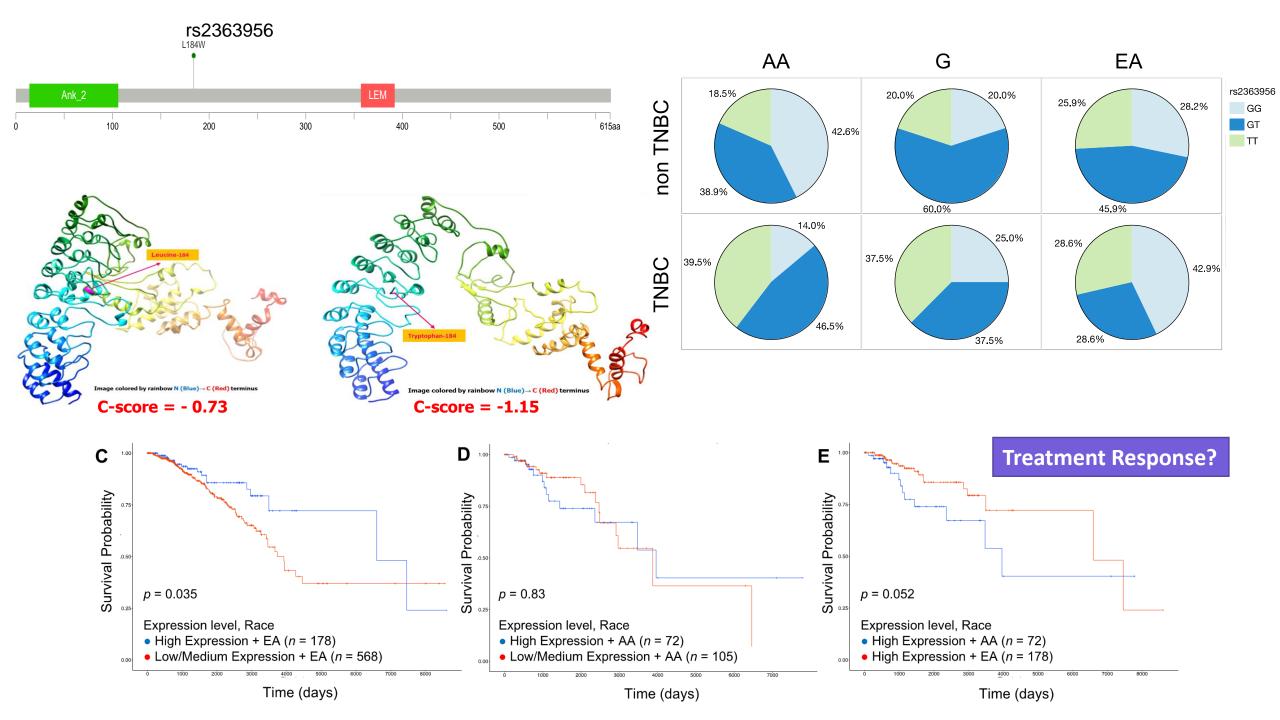# TNBC case-series risk analysis of SNVs previously identified as BC risk alleles

Variant	Region	Associated/Neighboring Gene(s)
rs2814778	1q23	DARC/ACKR1
rs13000023	2q35	TNP1, DIRC3
rs2363956	19p13.11	ANKLE1
rs2981578	10q26	FGFR2
rs2981579	10q26.13	FGFR2
rs3112572	16q12	LOC643714
rs3745185	19p13	BABAM1
rs4245739	1q32.1	MDM4
rs4849887	2q14.2	LOC84931, GLI2
rs609275	11q13	MYEOV,CCND1

rs2363956
L184W

Ank_2   LEM

C-score = - 0.73   C-score = -1.15

Image colored by rainbow N (Blue)→ C (Red) terminus

Leucine-184   Tryptophan-184

AA   G   EA

rs2363956
GG
GT
TT

non TNBC

18.5%   42.6%   20.0%   20.0%   25.9%   28.2%
38.9%   60.0%   45.9%

TNBC

14.0%   25.0%   28.6%   42.9%
39.5%   46.5%   37.5%   37.5%   28.6%

C   D   E   Treatment Response?

p = 0.035   *p* = 0.83   *p* = 0.052

Expression level, Race
High Expression + EA (*n* = 178)
Low/Medium Expression + EA (*n* = 568)

Expression level, Race
High Expression + AA (*n* = 72)
Low/Medium Expression + AA (*n* = 105)

Expression level, Race
High Expression + AA (*n* = 72)
High Expression + EA (*n* = 178)

African-specific BRCA1/2 Alleles

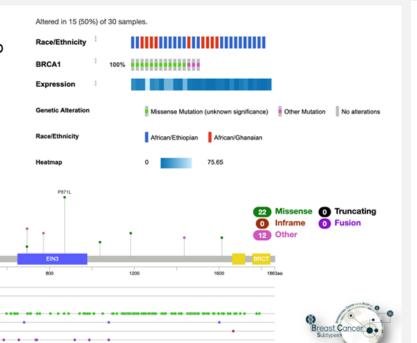Assessing functional consequences

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Premise: Taking SNP calls used for ancestry quantification, can we detect BRCA1/2 [somatic] mutations in our African and domestic RNAseq TNBC cohorts?
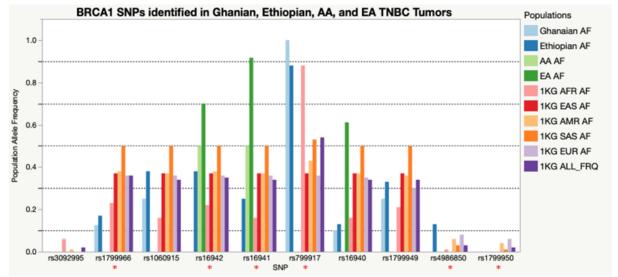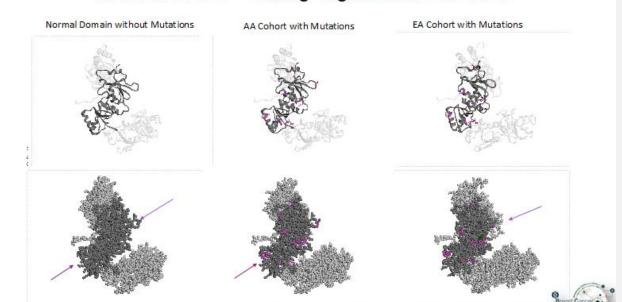
BRCA1 SNPs lollipop plot, domain structure, and heatmap for Ethiopian and Ghanaian cohorts

BRCA1 SNPs in Ghanaian, Ethiopian, AA, and EA TNBC tumors compared against 1000 Genome super group populations, ordered by CHR position

BRCA1 SNPs – Giving 'significance' to VUS

Normal Domain without Mutations

AA Cohort with Mutations

EA Cohort with Mutations

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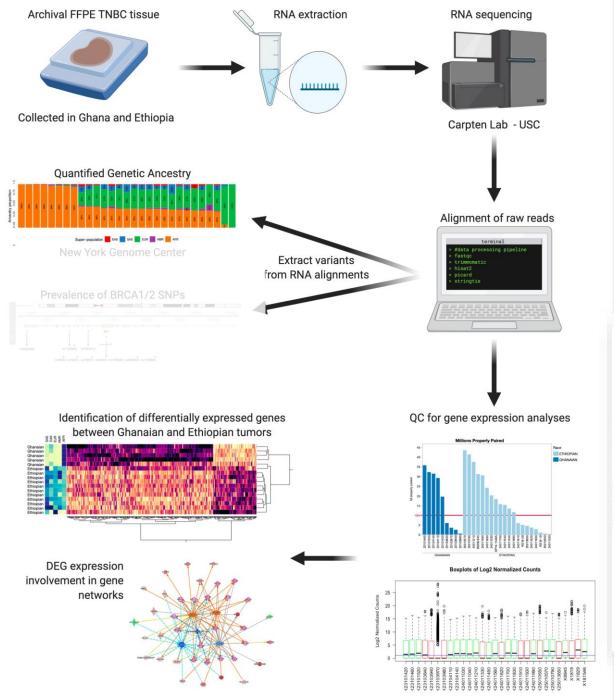
BRCA2 SNPs identified in Ghanaian and Ethiopian TNBC tumors (from RNAseq)

SNP rsID	Variant Type	AA Change	SIFT / Polyphen	Clinical Significance	Ghanaian AF		Ethiopian AF		1KG AFR AF	1KG Global AF
rs1799943	5 Prime UTR			Benign	0.00	*n* = 1	-	-	0.23	0.21
rs766173	Missense	N289H	Deleterious / Possibly Damaging	Likely benign / Conflicting Interpretations of Pathogenicity	-	-	0.00	*n* = 3	0.07	0.07
rs144848	Missense	N372H	Tolerated / Possibly Damaging	Benign / Uncertain significance	0.13	*n* = 4	0.30	*n* = 7	0.31	0.25
rs1801439	Synonymous	S455S		Benign	0.00	*n* = 2	0.00	*n* = 4	0.07	0.07
rs1801499	Synonymous	H743H		Benign	0.00	*n* = 5	0.00	*n* = 9	0.07	0.07
rs1799944	Missense	N991D	Tolerated / Benign	Benign	0.00	*n* = 1	0.00	*n* = 2	0.07	0.08
rs1801406	Synonymous	K1132K			0.00	*n* = 3	0.10	*n* = 14	0.27	0.27
rs543304	Synonymous	V1269V		Likely benign	0.00	*n* = 2	0.00	*n* = 4	0.17	0.17
rs206075	Synonymous	L4563L		Likely benign	1.00	*n* = 5	1.00	*n* = 3	1.00	0.97
rs1799955	Synonymous	S2414S		Likely benign	0.00	*n* = 1	0.40	*n* = 4	0.22	0.23
rs169547	Missense	V2466A	Tolerated / Benign	Uncertain significance	-	-	1.00	*n* = 1	1.00	0.98
rs9590940	Synonymous	V2820V		Likely benign	0.00	*n* = 5	0.00	*n* = 5	0.00	0.01
rs1801426	Downstream / Missense	I3412V	Tolerated / Benign	Uncertain significance	0.00	*n* = 1	0.30	*n* = 2	0.02	0.04

Ancestry-Associated Gene Network Changes

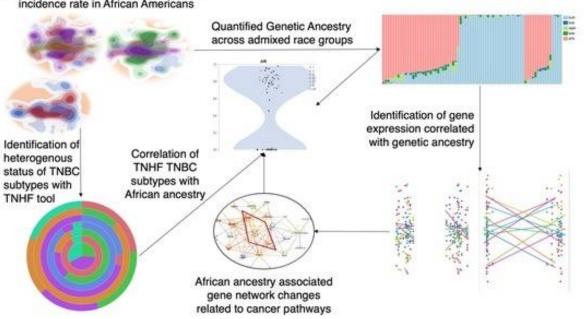Power of Inclusion

Melissa B Davis, PhD

Identification of Distinct Heterogenic Subtypes and Molecular Signatures Associated with African Ancestry in Triple Negative Breast Cancer Using Quantified Genetic Ancestry Models in Admixed Race Populations
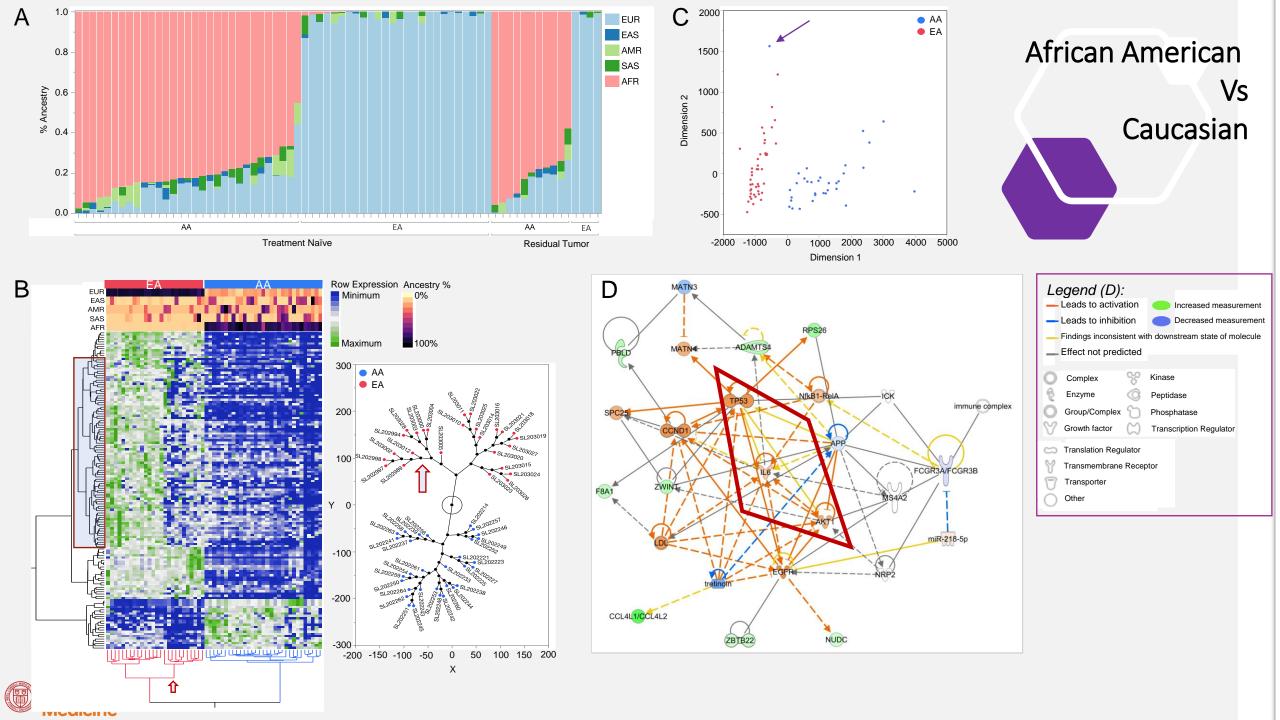
by Melissa Davis [1,†] ✉ 🄾, Rachel Martini [1,†] ✉, Lisa Newman [1] ✉, Olivier Elemento [2,3,4] ✉, Jason White [5] ✉, Akanksha Verma [6] ✉, Indrani Datta [7] ✉, Indra Adrianto [7] ✉, Yalei Chen [7] ✉, Kevin Gardner [8] ✉, Hyung-Gyoon Kim [9] ✉, Windy D. Colomb [5,10] ✉, Isam-Eldin Eltoum [9,11] ✉, Andra R. Frost [9,11] ✉, William E. Grizzle [9,11,12] ✉, Andrea Sboner [3,4,13] ✉, Upender Manne [9,11,*] ✉ and Clayton Yates [5,*] ✉ 🄾

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[3] Caryl and Israel Englander Institute for Precision Medicine, Weill Cornell Medicine, New York, NY 10065, USA
[4] Institute for Computational Biomedicine, Weill Cornell Medicine, New York, NY 10065, USA
[5] Department of Biology and Center for Cancer Research, Tuskegee University, Tuskegee, AL 36088, USA
[6] Department of Computational Biology, Weill Cornell Medicine, New York, NY 10065, USA
[7] Department of Public Health Sciences, Henry Ford Health System, Detroit, MI 48202, USA
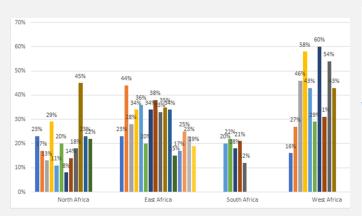
A

B

C

African American
Vs
Caucasian

D

Legend (D):
Leads to activation — Increased measurement
Leads to inhibition — Decreased measurement
Findings inconsistent with downstream state of molecule
Effect not predicted

Complex — Kinase
Enzyme — Peptidase
Group/Complex — Phosphatase
Growth factor — Transcription Regulator
Translation Regulator
Transmembrane Receptor
Transporter
Other
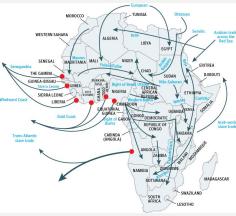
Pan-African Comparisons
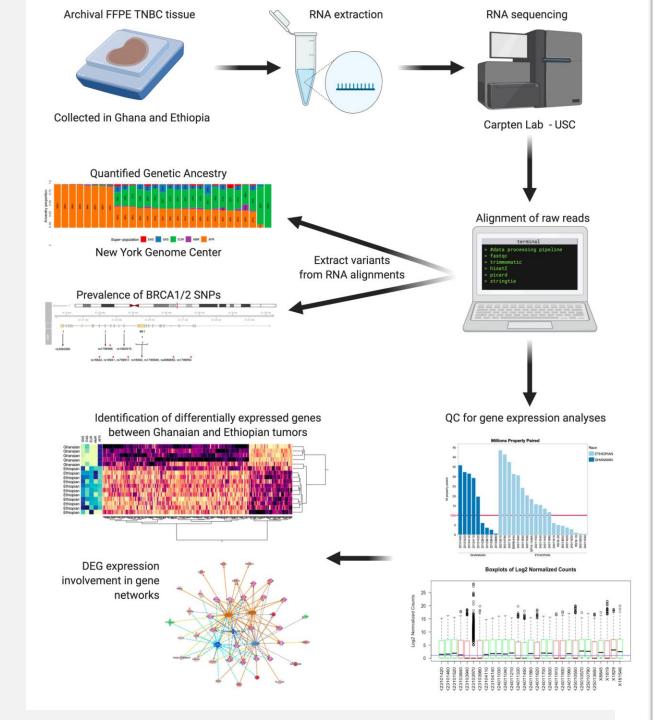
RNA sequencing of TNBC tumors from Ghanaian and Ethiopian women
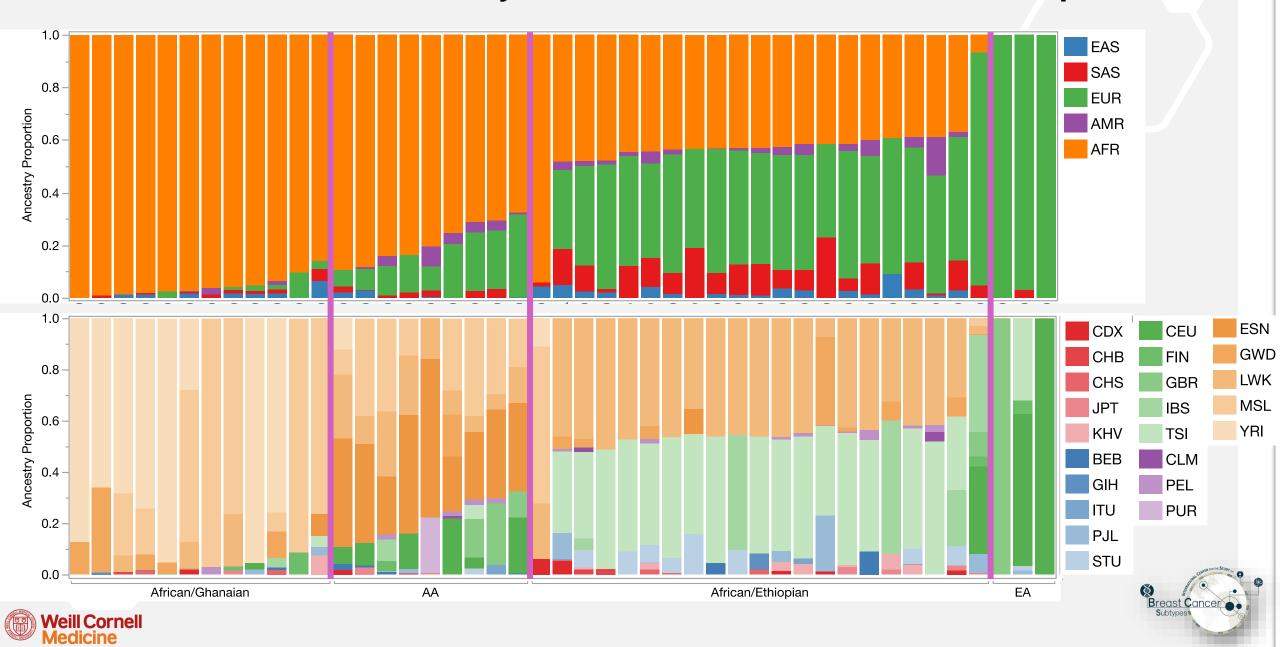
1. Ancestry Gene Network Function
2. Novel mutations in Cancer Genes
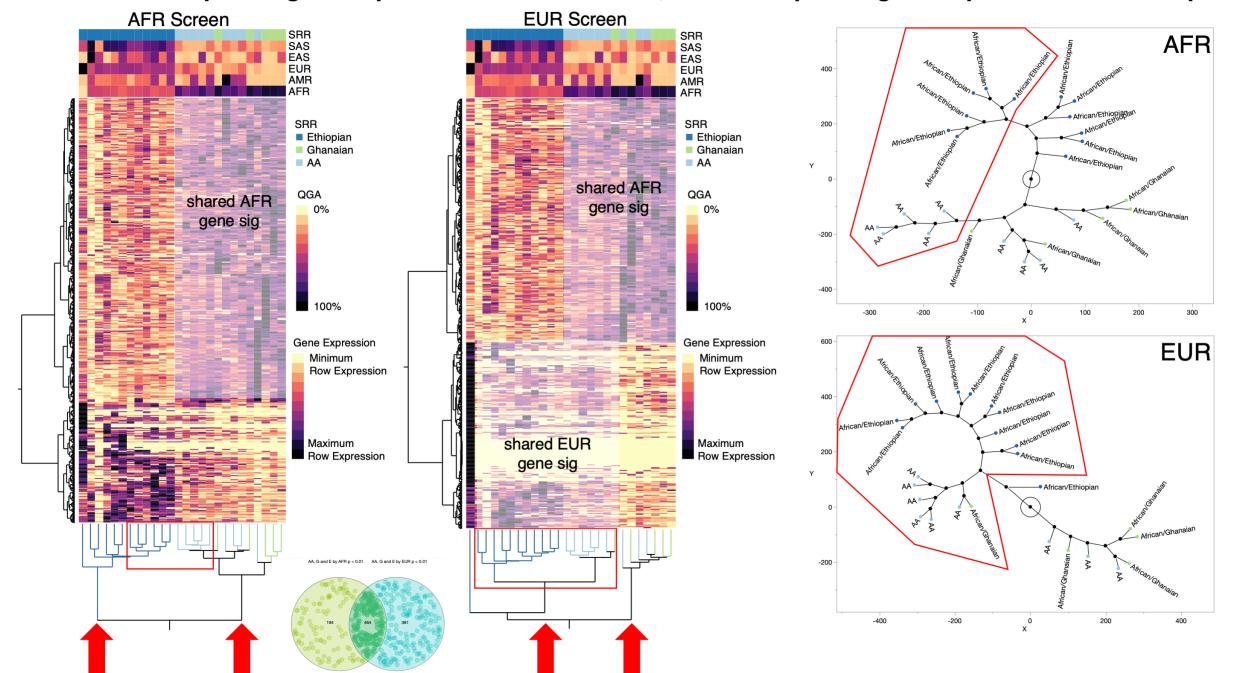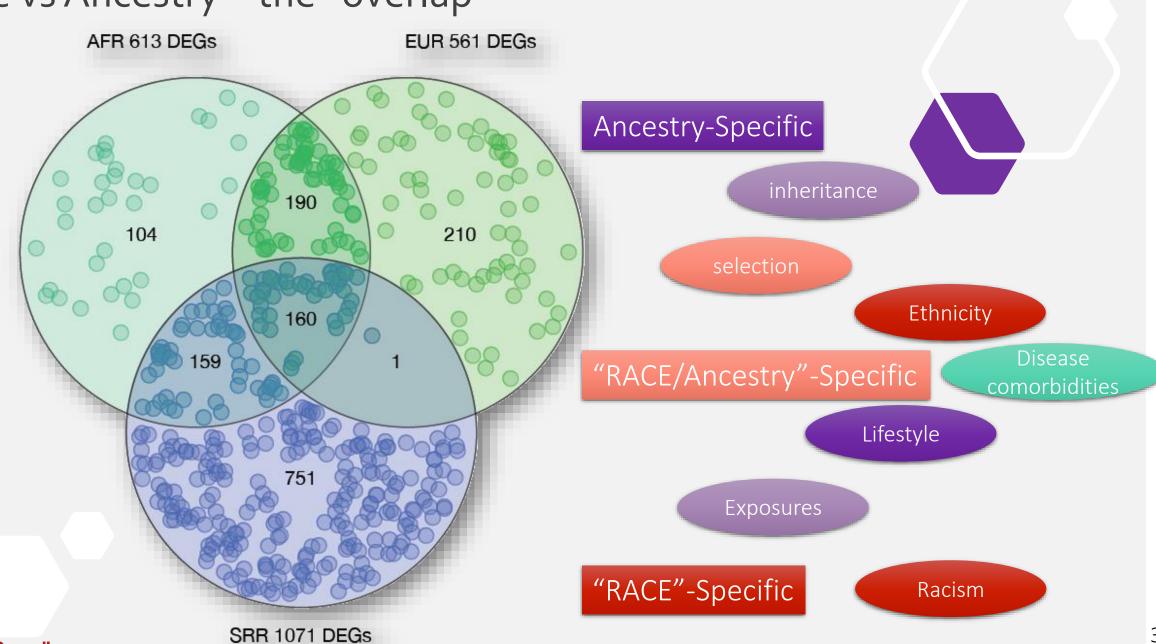
Quantified Genetic Ancestry Estimations in our ICSBCS RNAseq Cohort

AAs share AFR-specific gene expression with Ghanaians, and EUR-specific gene expression with Ethiopians
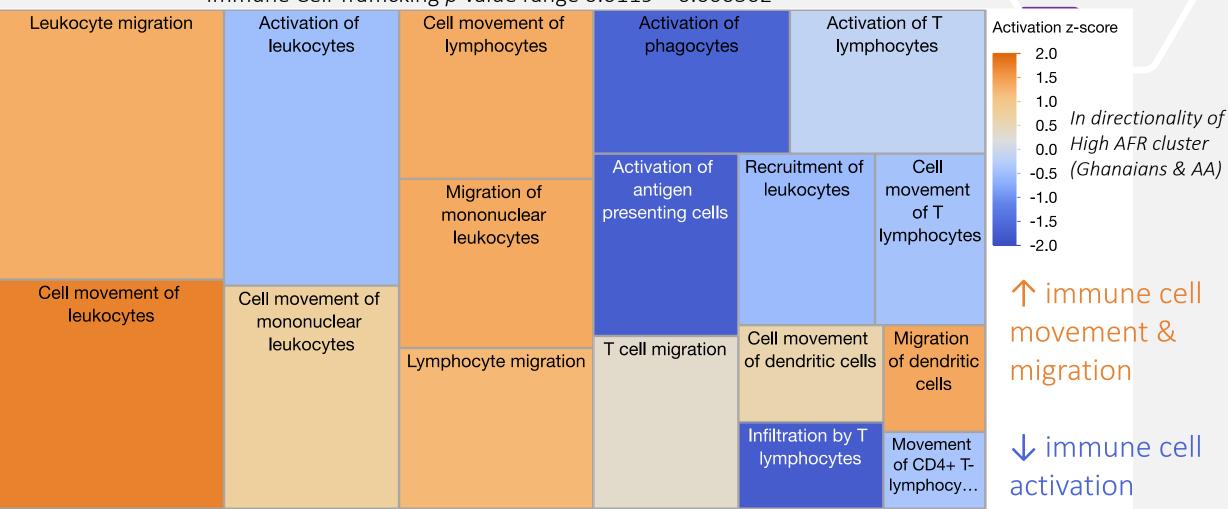
Race vs Ancestry – the "overlap"

Melissa B Davis, PhD

Increase in immune cell movement and migration, but not activation, associated with High AFR cluster genes
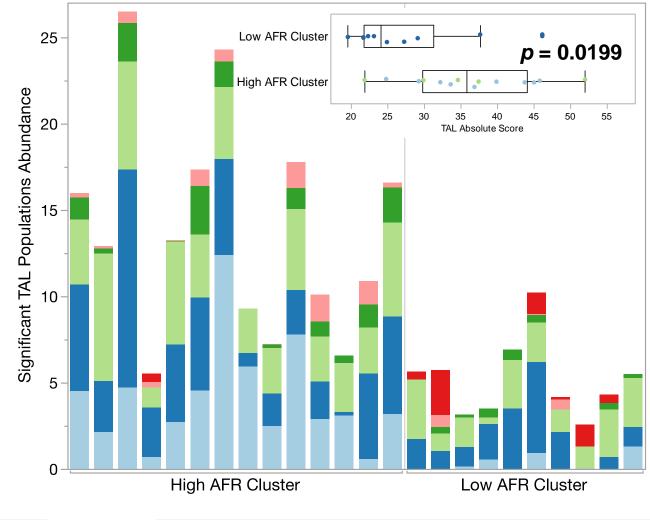
Immune Cell Trafficking *p* value range 0.0119 – 0.000502

Diseases or Functions Annotation

Activation z-score

In directionality of High AFR cluster (Ghanaians & AA)

↑ immune cell movement & migration

↓ immune cell activation

Abundance of tumor-associated leukocytes (TAL) significantly higher among High AFR cluster

Deconvolution using CIBERSORTx

TAL population	High AFR cluster mean	Low AFR cluster mean	Student's t-test *p* value	*ANOVA* by SRR *p* value
B cells naive	**4.12**	0.29	0.0004	0.0016
T cells CD8	**4.16**	1.87	0.0268	0.0248
T cells follicular helper	**3.98**	1.98	0.0019	0.0037
NK cells activated	**1.01**	0.27	0.0103	0.0455
Monocytes	**0.55**	0.14	0.0291	0.0403
Mast cells activated	0.02	**0.61**	0.0613	0.0665

AA vs EA TNBC Immune Profiles
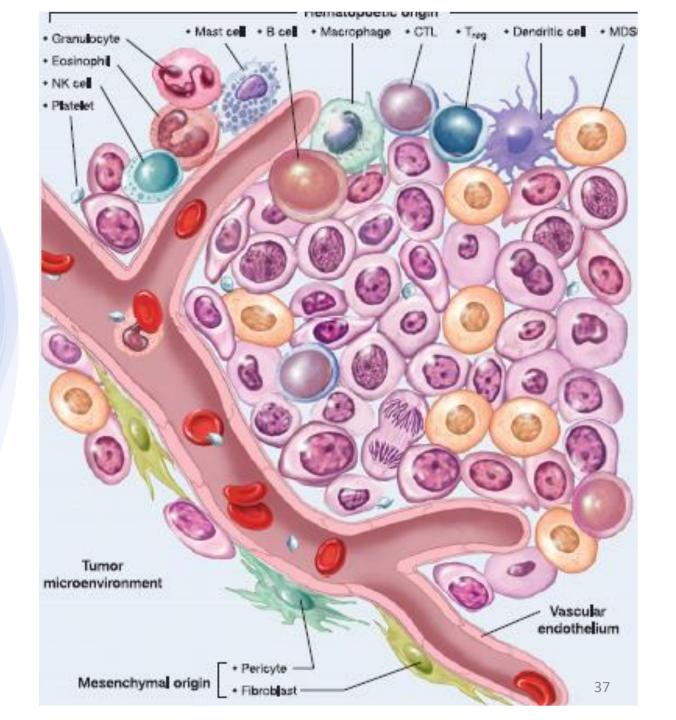
Davis, M.; Martini, R.; Newman, L.; Elemento, O.; White, J.; Verma, A.; Datta, I.; Adrianto, I.; Chen, Y.; Gardner, K.; Kim, H.-G.; Colomb, W.D.; Eltoum, I.-E.; Frost, A.R.; Grizzle, W.E.; Sboner, A.; Manne, U.; Yates, C. Identification of Distinct Heterogenic Subtypes and Molecular Signatures Associated with African Ancestry in Triple Negative Breast Cancer Using Quantified Genetic Ancestry Models in Admixed Race Populations. Cancers 2020, 12, 1220.

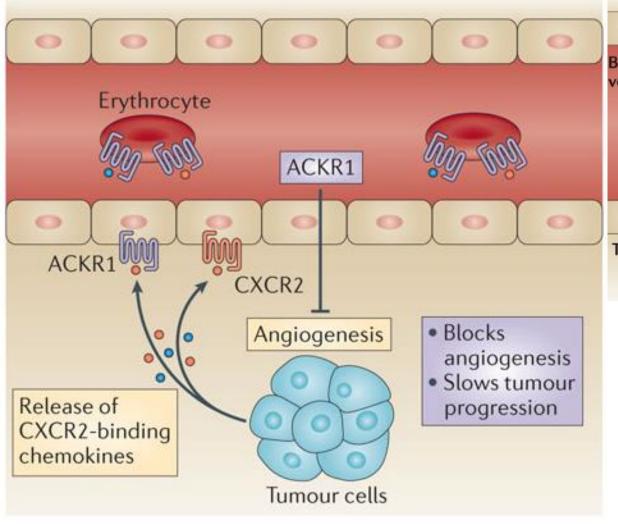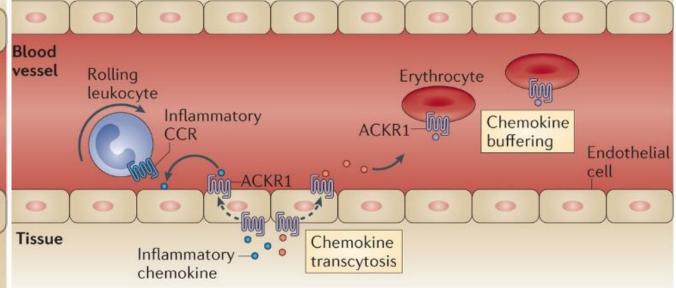# Tumor Immunology –

Evolution at Work

Melissa B Davis, PhD
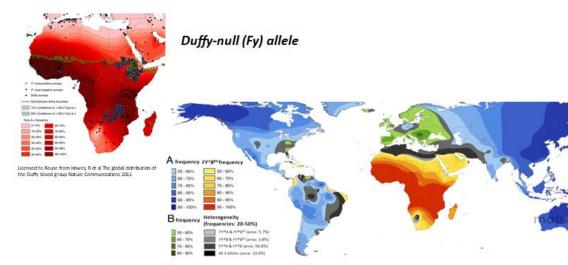
DARC Immune Tumor Phenotype

Unique immune response – ancestry specific

DARC regulates dynamic inflammatory chemokine levels via vascular endothelial cells and concentrations in plasma
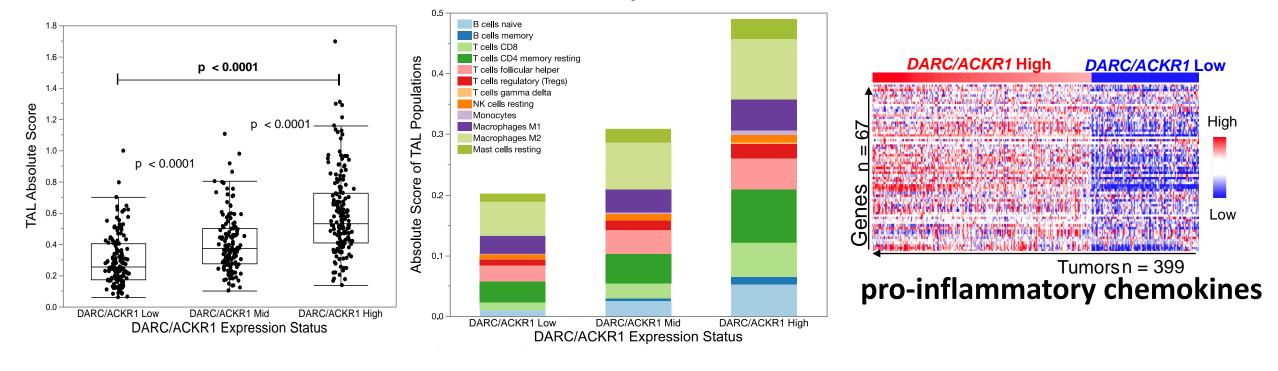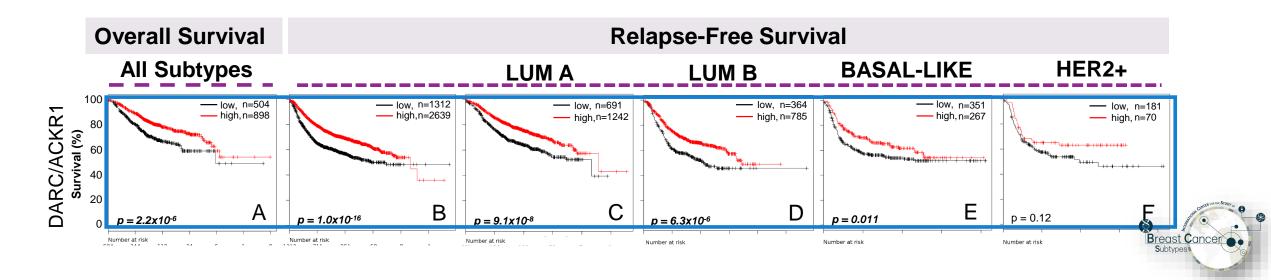

Nature Reviews | Immunology

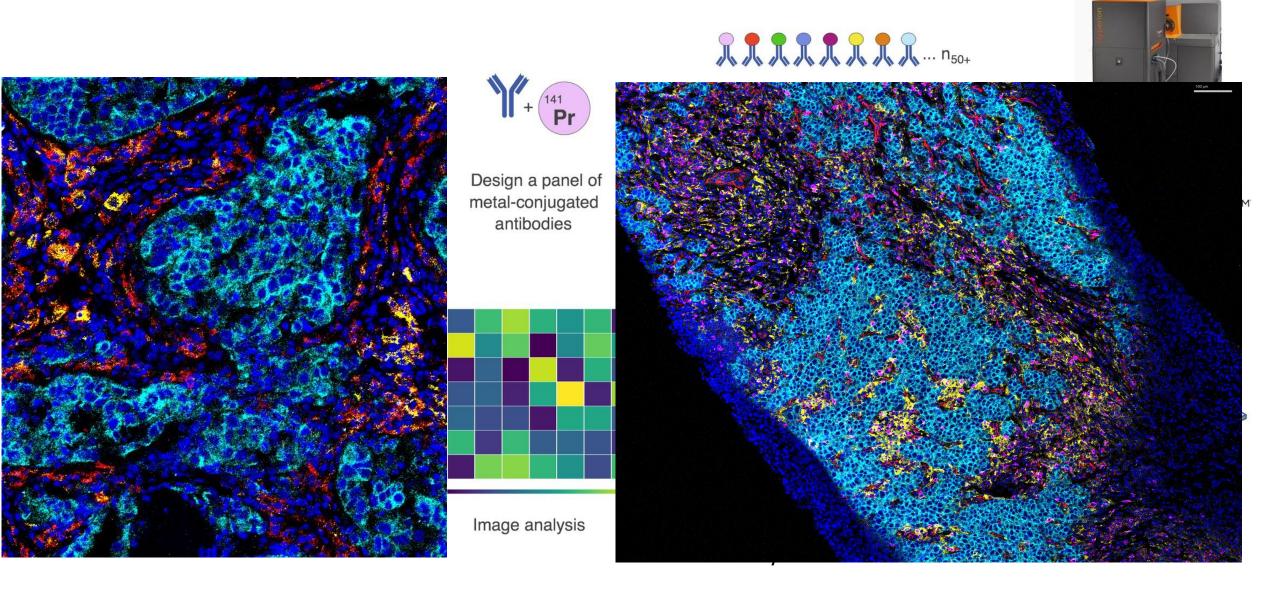*Nibbs RJ, Graham GJ. Immune regulation by atypical chemokine receptors. Nat Rev Immunol. 2013*

DARC-associated tumor leukocyte associations in TCGA cohort

Imaging mass cytometry to define spatial distinctions
Characterizing the DARC immuno-tumor-type

Design a panel of metal-conjugated antibodies
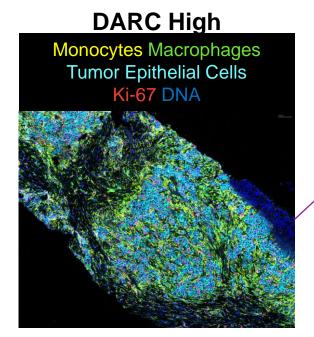
Image analysis

TNBC tumors (n=4)

DARC high by IHC (n=2)
2x ROI from case = 4 ROI total

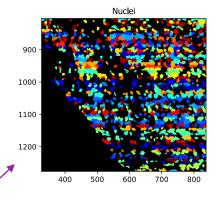DARC low by IHC (n=2)
2x ROI from case = 4 ROI total

Hyperion™ IMC staining of ~30 markers
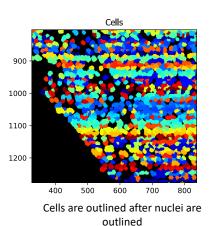Cell segmentation
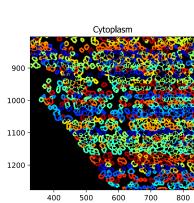histoCAT™ single-cell protein analysis (meta - tSNE & phenograph clustering)

DARC Low

Tumor-associated Macrophages
Tumor Epithelial Cells
Monocytes DNA

DARC High

Monocytes Macrophages
Tumor Epithelial Cells
Ki-67 DNA

Nuclei

Cells

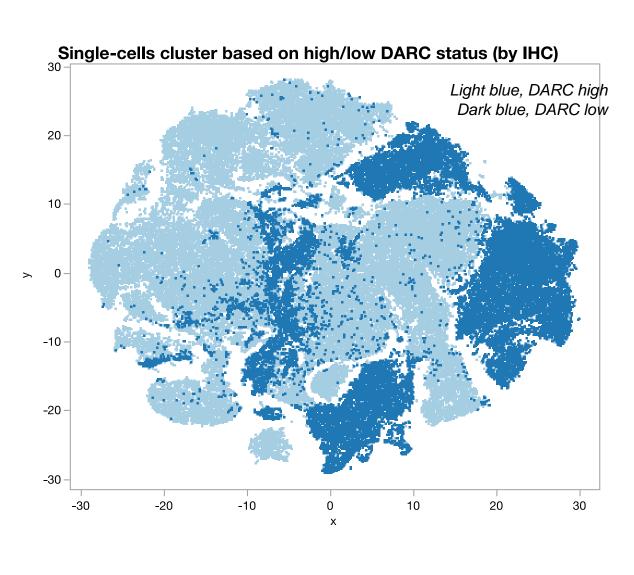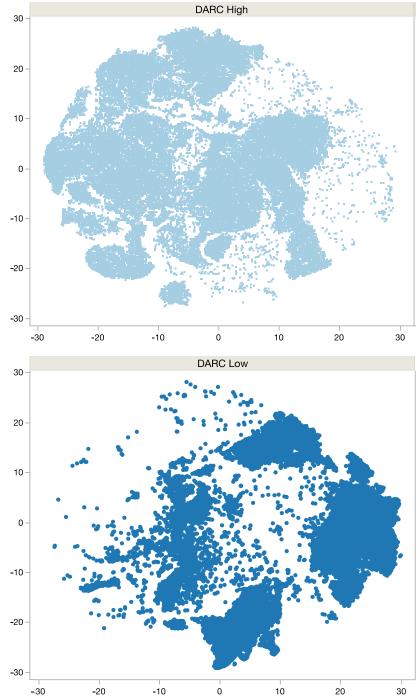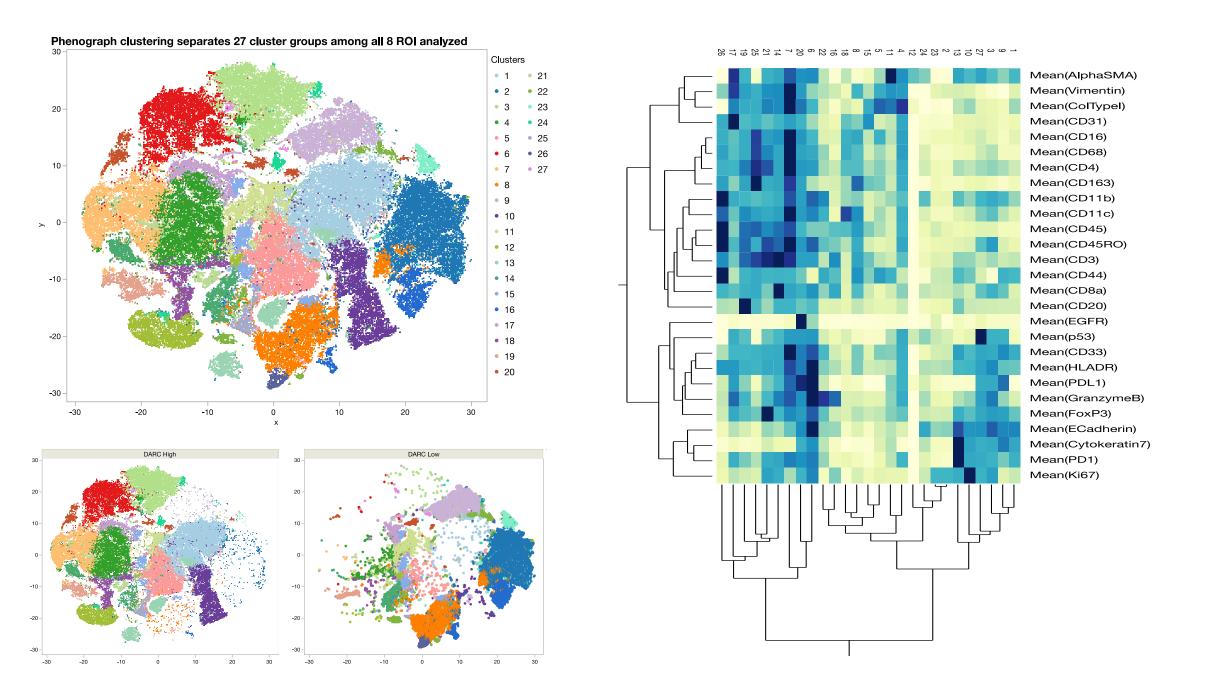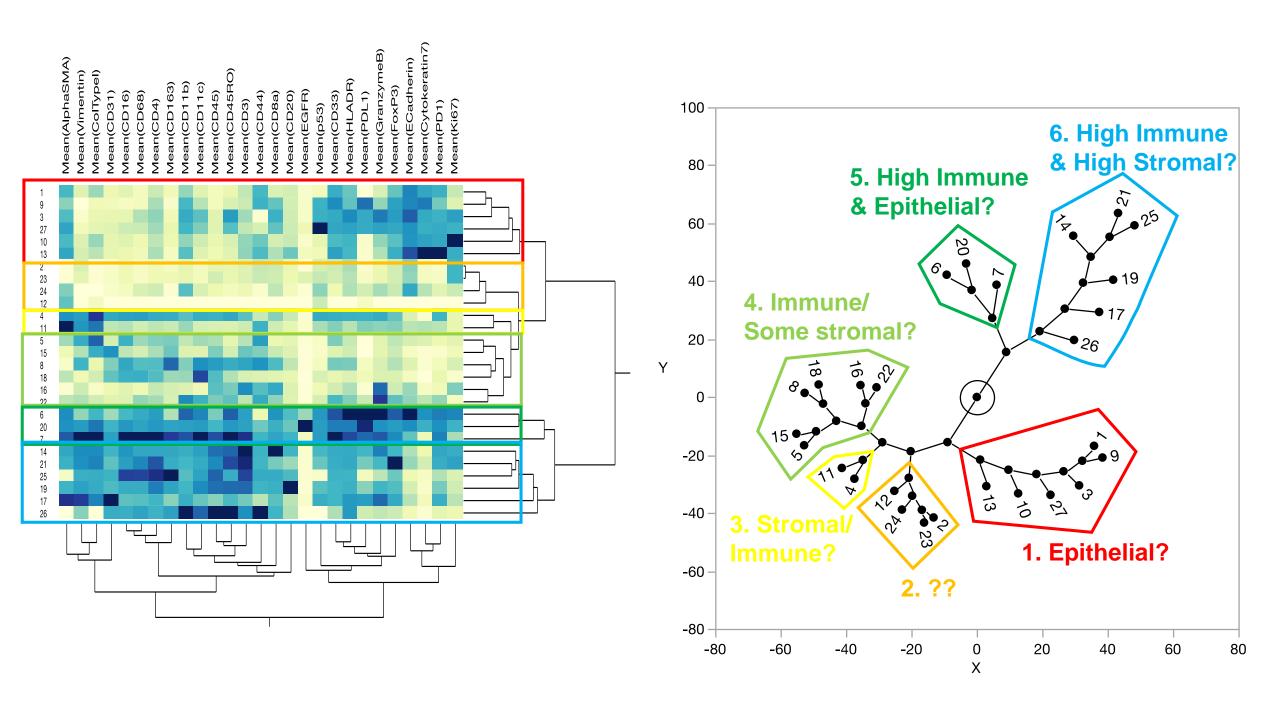Cells are outlined after nuclei are outlined

Cytoplasm

Cytoplasmic area is identified as the space b/t the nuclei and the cellular outline

DARC-specific clusters of tumor-associated cells

Single-cells cluster based on high/low DARC status (by IHC)

Light blue, DARC high
Dark blue, DARC low

Phenograph clustering separates 27 cluster groups among all 8 ROI analyzed

Column labels (left heatmap, top to bottom): Mean(AlphaSMA), Mean(Vimentin), Mean(ColTypeI), Mean(CD31), Mean(CD16), Mean(CD68), Mean(CD4), Mean(CD163), Mean(CD11b), Mean(CD11c), Mean(CD45), Mean(CD45RO), Mean(CD3), Mean(CD44), Mean(CD8a), Mean(CD20), Mean(EGFR), Mean(p53), Mean(CD33), Mean(HLADR), Mean(PDL1), Mean(GranzymeB), Mean(FoxP3), Mean(ECadherin), Mean(Cytokeratin7), Mean(PD1), Mean(Ki67)

Right panel labels:

6. High Immune & High Stromal?

5. High Immune & Epithelial?

4. Immune/ Some stromal?

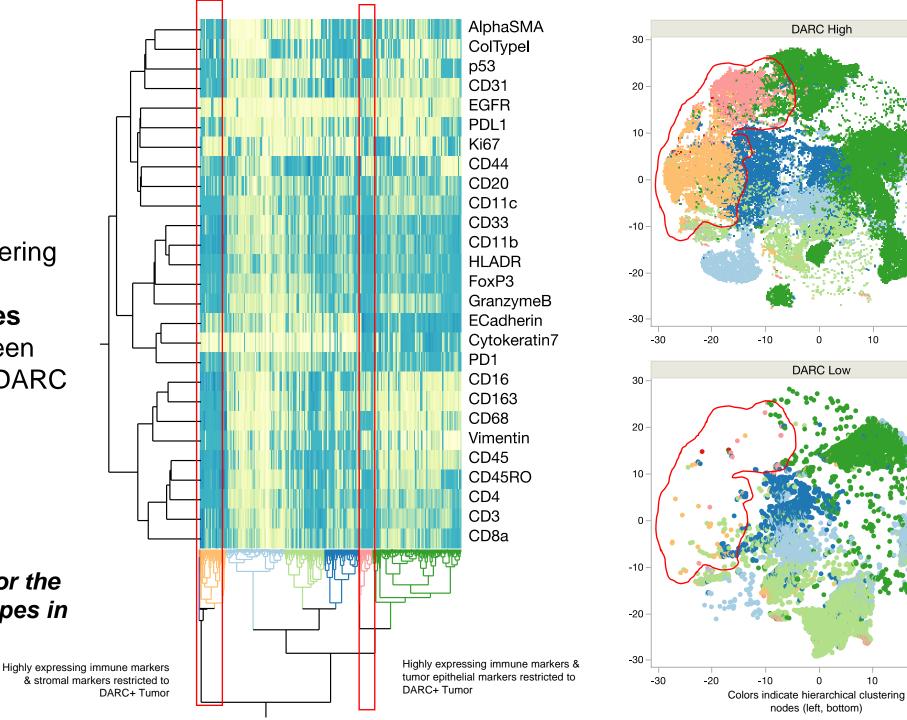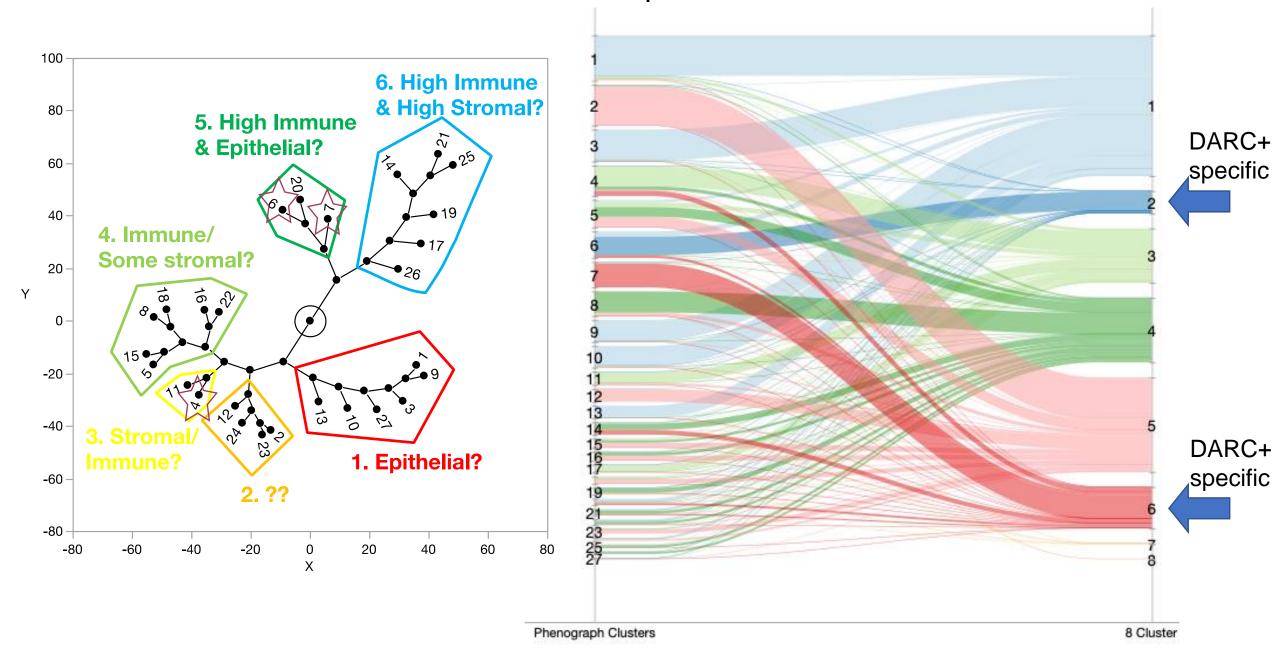3. Stromal/ Immune?

2. ??

1. Epithelial?

- Hierarchical clustering **functionally descriptive nodes**
- distinctions between DARC High and DARC low tumors
- **7 distinct nodes**

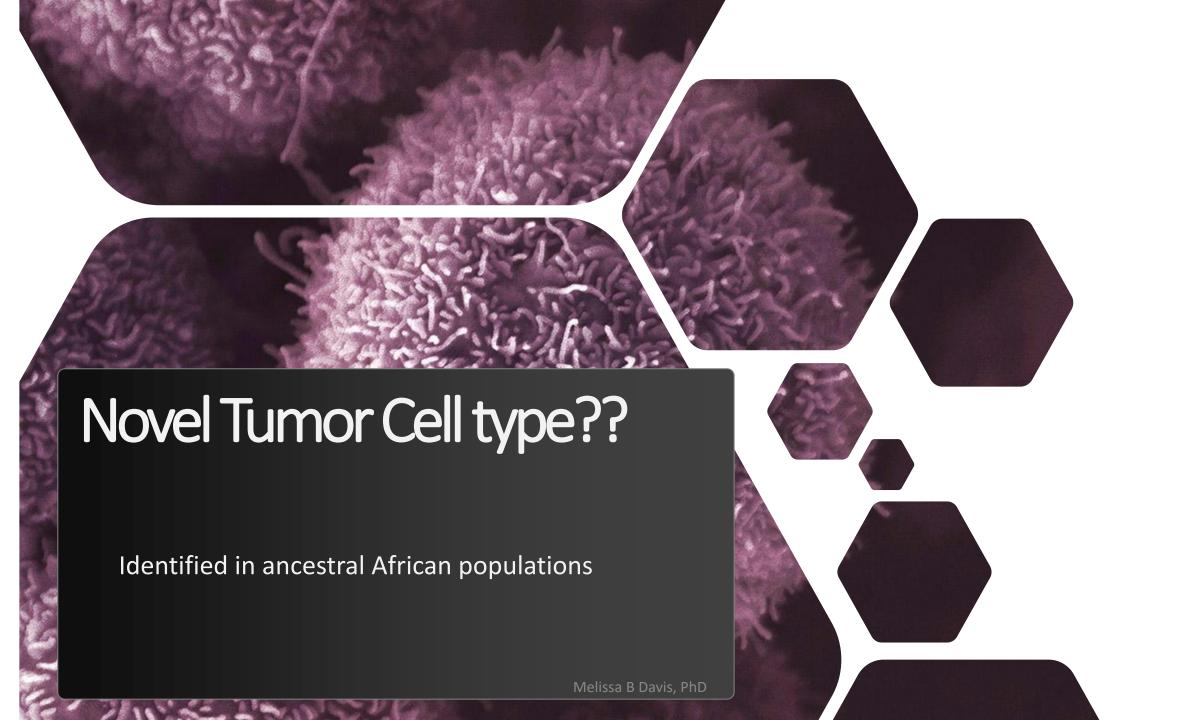*Next steps… Look for the 'new DARC phenotypes in tissues*

Highly expressing immune markers & stromal markers restricted to DARC+ Tumor

Highly expressing immune markers & tumor epithelial markers restricted to DARC+ Tumor

AlphaSMA
ColTypeI
p53
CD31
EGFR
PDL1
Ki67
CD44
CD20
CD11c
CD33
CD11b
HLADR
FoxP3
GranzymeB
ECadherin
Cytokeratin7
PD1
CD16
CD163
CD68
Vimentin
CD45
CD45RO
CD4
CD3
CD8a

DARC High

DARC Low

Colors indicate hierarchical clustering nodes (left, bottom)

Tumor-associated cells that are DARC+ specific?

Novel Tumor Cell type??

Identified in ancestral African populations
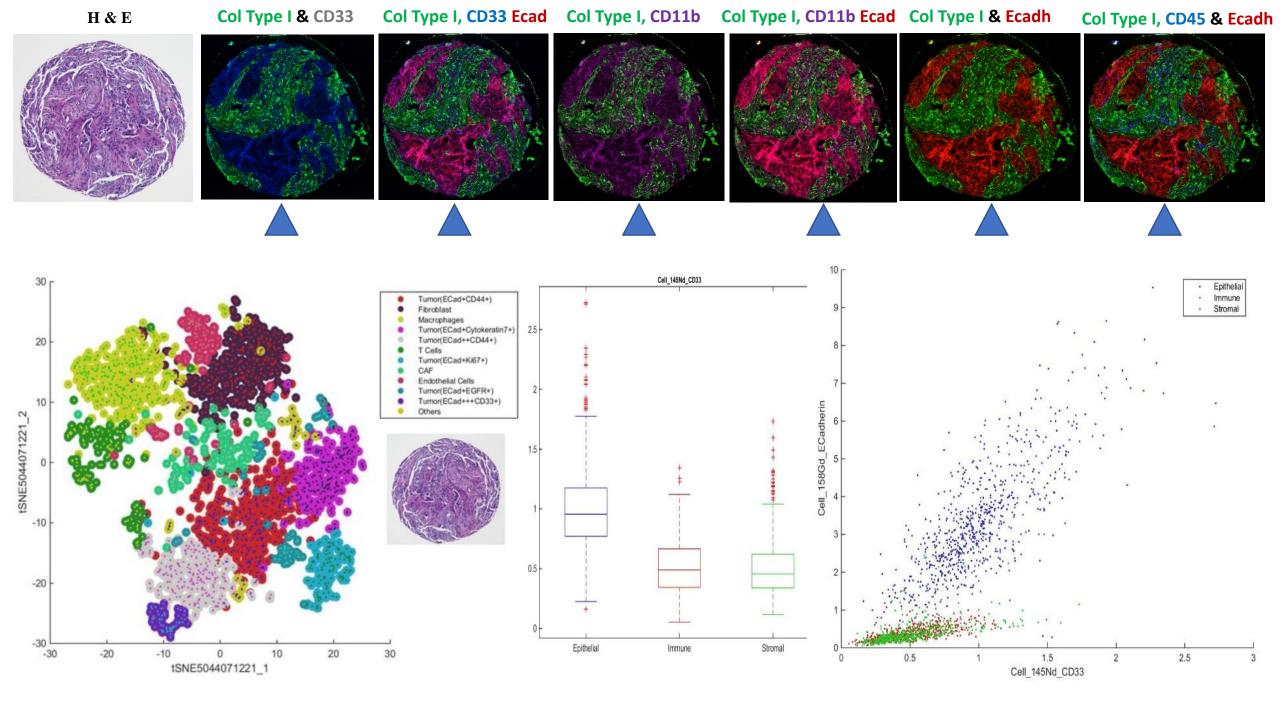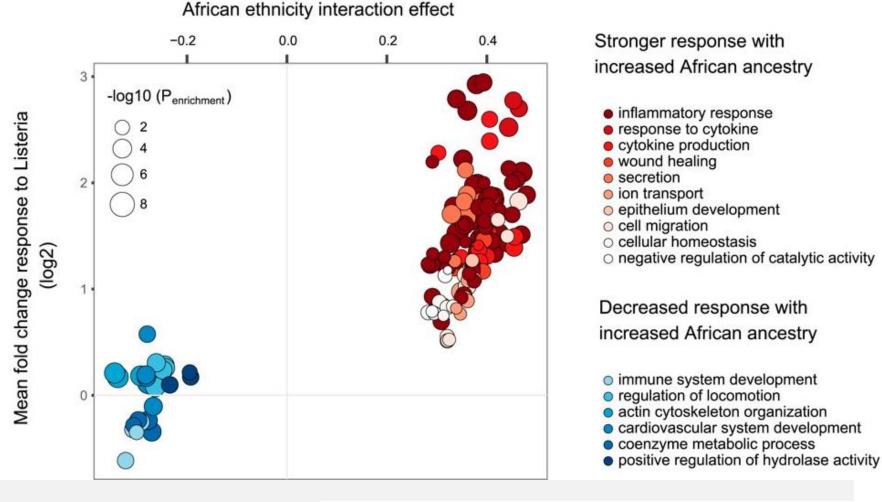
Ethiopian TMA

- Combination of Tumor Subtypes
- Investigation of tumor heterogeneity
- Distinctions associated with ancestry

H & E

Col Type I & CD33

Col Type I, CD33 Ecad

Col Type I, CD11b

Col Type I, CD11b Ecad

Col Type I & Ecadh

Col Type I, CD45 & Ecadh

Tumor(ECad+CD44+)
Fibroblast
Macrophages
Tumor(ECad+Cytokeratin7+)
Tumor(ECad++CD44+)
T Cells
Tumor(ECad+Ki67+)
CAF
Endothelial Cells
Tumor(ECad+EGFR+)
Tumor(ECad+++CD33+)
Others

Cell_145Nd_CD33

Epithelial   Immune   Stromal

Epithelial
Immune
Stromal

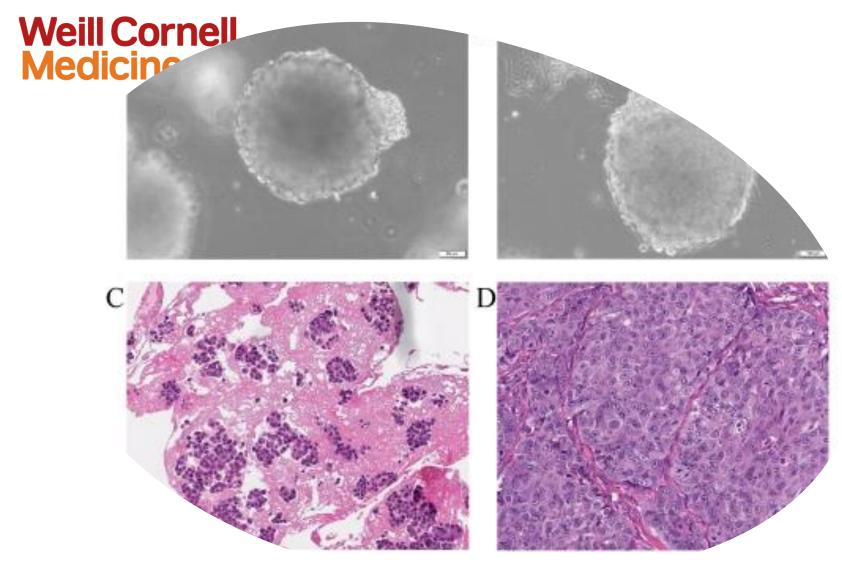# Rationale: Distinct Immune Response – Evolution Influences

Human evolution genetics

- African Ancestry is associated with a stronger immune response
- Specific responses include wound healing, cytokine production and inflammatory response

African ethnicity interaction effect

Mean fold change response to Listeria (log2)

$-log10 (P_{enrichment})$
- 2
- 4
- 6
- 8

Stronger response with increased African ancestry
- inflammatory response
- response to cytokine
- cytokine production
- wound healing
- secretion
- ion transport
- epithelium development
- cell migration
- cellular homeostasis
- negative regulation of catalytic activity

Decreased response with increased African ancestry
- immune system development
- regulation of locomotion
- actin cytoskeleton organization
- cardiovascular system development
- coenzyme metabolic process
- positive regulation of hydrolase activity

Modified From: Nedelec, Y et. al, *Genetic Ancestry and Natural Selection Drive Population Differences in Immune Responses to Pathogens.* Cell, 2016. 167(3): p. 657-669 e21.
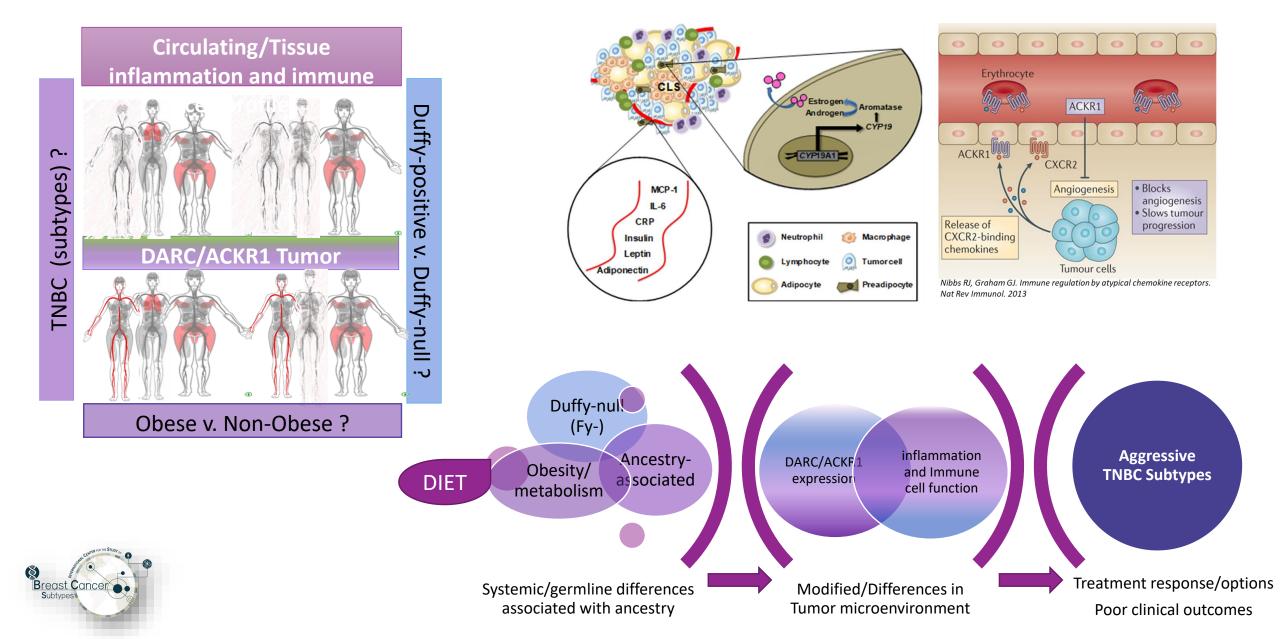
Weill Cornell Medicine

Future Directions Breast Cancer Disparities

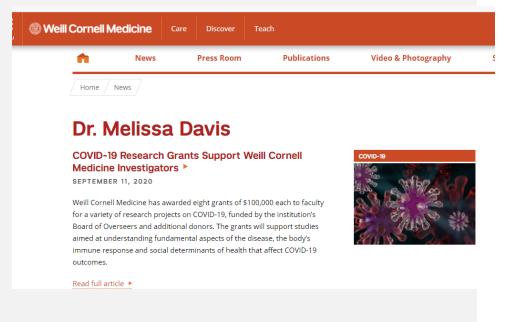*Artificial Intelligence, In vivo models, Ex vivo screens and global clinical genomics*

DARC STARR Premise : DARC-regulated Race-group TME differences
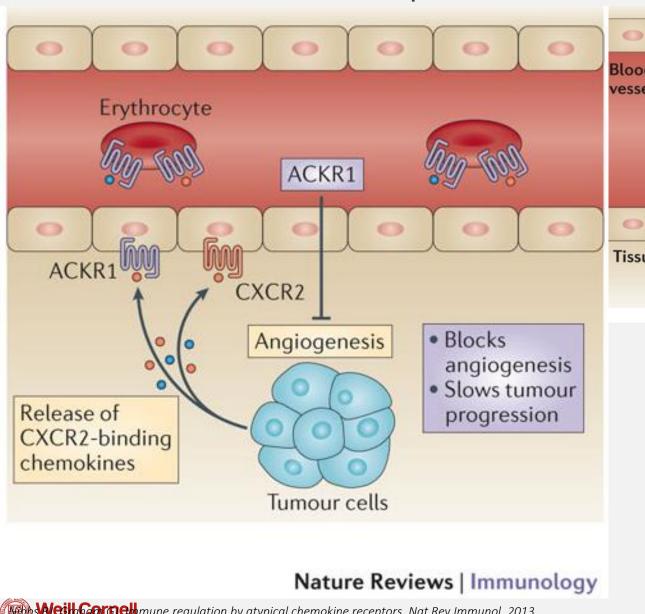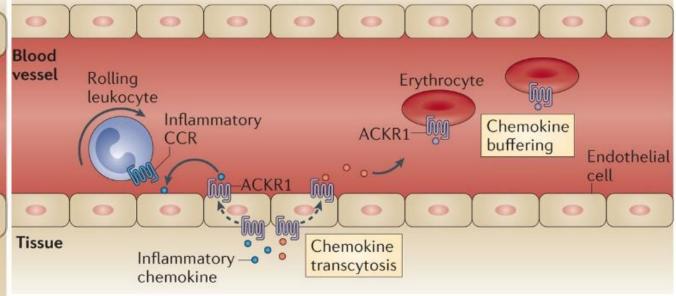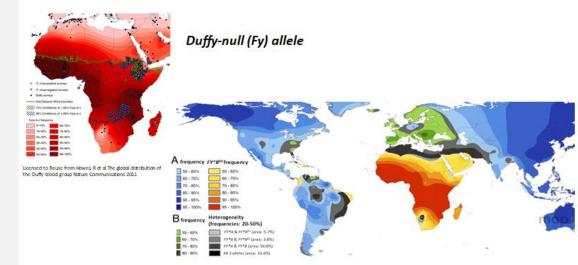## *Obesity-related? *Ancestry-related? *Duffy-null(Fy-)-regulated?

Circulating/Tissue inflammation and immune

DARC/ACKR1 Tumor

TNBC (subtypes) ?

Duffy-positive v. Duffy-null ?

Obese v. Non-Obese ?

CLS

Estrogen, Androgen → Aromatase → CYP19

CYP19A1

MCP-1
IL-6
CRP
Insulin
Leptin
Adiponectin

Neutrophil   Macrophage
Lymphocyte   Tumor cell
Adipocyte   Preadipocyte

Erythrocyte

ACKR1

ACKR1   CXCR2

Angiogenesis

- Blocks angiogenesis
- Slows tumour progression

Release of CXCR2-binding chemokines

Tumour cells

Nibbs RJ, Graham GJ. Immune regulation by atypical chemokine receptors. Nat Rev Immunol. 2013

DIET

Duffy-null (Fy-)

Obesity/ metabolism

Ancestry-associated

DARC/ACKR1 expression

inflammation and Immune cell function

Aggressive TNBC Subtypes

Systemic/germline differences associated with ancestry

Modified/Differences in Tumor microenvironment

Treatment response/options

Poor clinical outcomes

Breast Cancer Subtypes

COVID-19 Impact

Exaggerating Disparities?

Melissa B Davis, PhD

DARC regulates dynamic inflammatory chemokine levels via vascular endothelial cells and concentrations in plasma
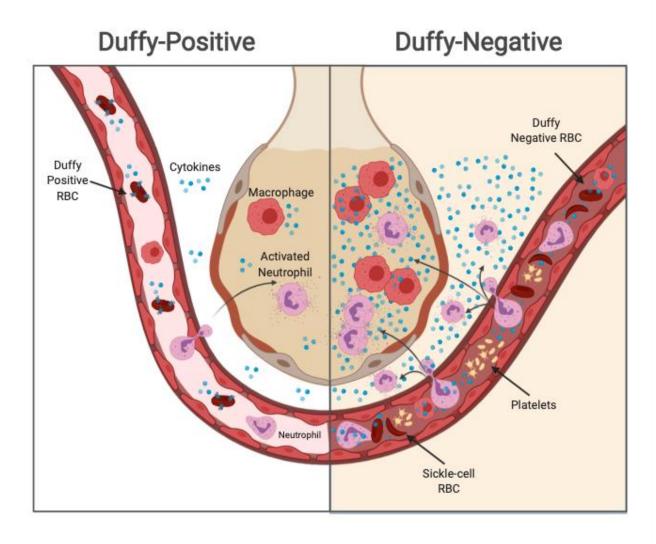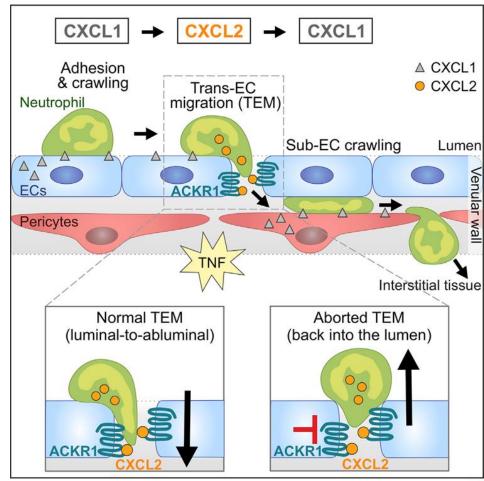

Nature Reviews | Immunology

Genetic imprint of the West African Diaspora
Impact on TNBC Risk?

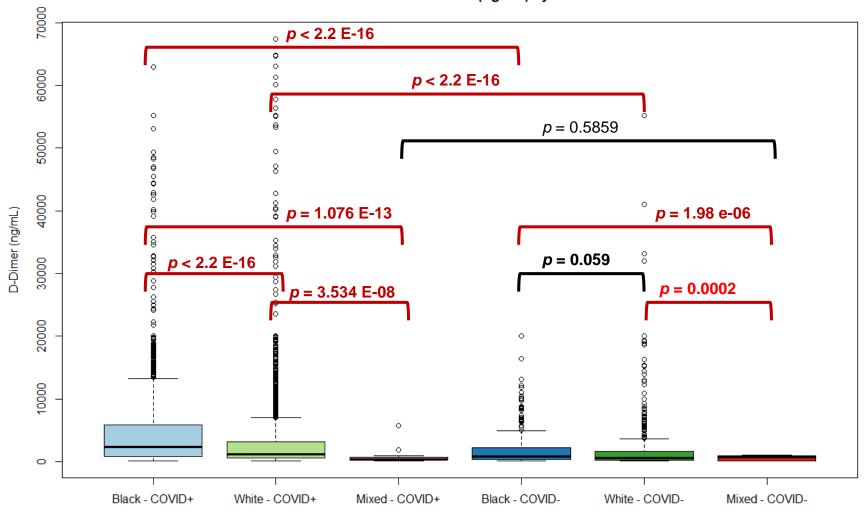Nibbs RJB, Graham GJ. Immune regulation by atypical chemokine receptors. Nat Rev Immunol. 2013

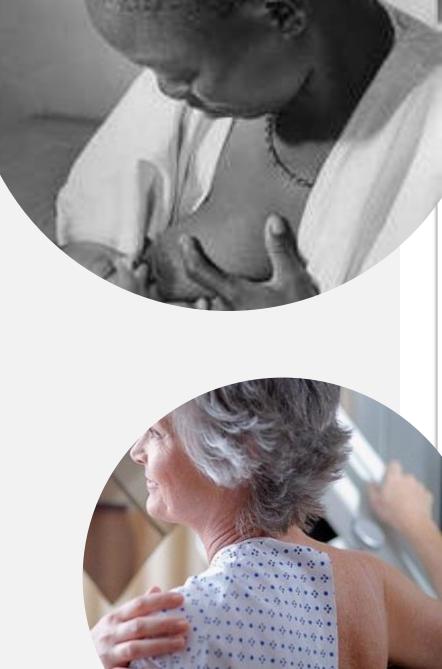# The DARC side of COVID-19 Disparities

Box Plots of D-Dimer (ng/mL) by Race

Summary

- Disparities in cancer mortality can be driven by biological distinctions

- INCLUSION of genetically diverse populations will reveal distinct mechanisms and/or functionality of genes involved in tumor progression

- Tumor immune response may drive race/ancestry group differences due to evolutionary adaptation across populations

- DARC tumor phenotypes have more infiltrating immune cells and distinct cell markers within immune cell groups

- Precision medicine tools can help identify new ways of treating disease phenotypes that are biased to certain populations

Acknowledgements...

Melissa B Davis, PhD

mbdavislab@gmail.com

Twitter: @melid32

Mbdavislab.org (coming soon)

DARC Immune Tumor Phenotype

Unique immune response – ancestry specific